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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 4 : C07K 7/18	A1	(11) International Publication Number: WO 89/ 09230
		(43) International Publication Date: 5 October 1989 (05.10.89)

(21) International Application Number: PCT/US89/01216

(22) International Filing Date: 22 March 1989 (22.03.89)

(31) Priority Application Number: 173,311

(32) Priority Date: 25 March 1988 (25.03.88)

(33) Priority Country: US

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(81) Designated States: AU, DK, FI, JP, NO.

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: THERAPEUTIC PEPTIDES

(57) Abstract

Bradykinin analogs containing a non-peptide bond; some of the analogs exhibit antagonist activity rendering useful therapeutically, e.g., for the relief of vasodilation - induced swelling and/or itching.

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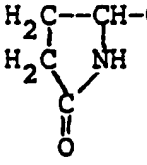
Therapeutic Peptides

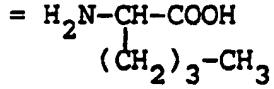
Background of the Invention

This invention relates to therapeutic peptides useful, e.g., in vasoconstriction.

Martinez et al. observed in studies on the mode of action of gastrin that changes in the peptide backbone (replacement of a carbonyl group with a methylene group) could provide analogues to gastrin with significantly altered biological activity, as agonists or antagonists (Martinez et al., J. Med. Chem., 28: 1874-1879 (1985)). Yet peptide bond replacement does not systematically provide for antagonist activity as Sasaki et al. found in a study of somatostatin analogues (Sasaki et al., J. Med. Chem., 30:1162-1166 (1987)), and Martinez et al. discovered in a study on the effect of changes to peptide bond of the C-terminal heptapeptide of cholecystokinin, a peptide which has much the same biological activity and the same final four amino acids as gastrin (Rodriquez et al., J. Med. Chem., 30:1366-1373 (1987)).

Abbreviations (uncommon):

pGlu =  (pyroglutamate);

Nle =  (norleucine)

Pal = 3-pyridyl-alanine

Nal = naphthylalanine

Summary of the Invention

In general, the invention features a bradykinin analog of the formula (1):

$$Q\text{-}A^1\text{-}A^2\text{-}A^3\text{-}Gly\text{-}A^5\text{-}A^6\text{-}A^7\text{-}A^8\text{-}A^9\text{-}Z^{10}$$

wherein

Q = L-Arg, D-Arg, L-homo-Arg, D-homo-Arg, L-Lys, D-Lys, lower (1-5 carbon atoms) ∑-N-alkyl-L-Lys, lower ∑-N-alkyl-D-Lys, lower ∑-N-alkyl-L-His, lower ∑-N-alkyl-D-His, lower ∑-N-alkyl-L-Pal, lower ∑-N-alkyl-D-Pal, acetyl, lower acyl, or lower α-N-alkyl;

A^1 and A^9 (independently) = Arg, homo-Arg, Lys, lower ∑-N-alkyl-Lys, His, or Pal;

A^2 = Pro, hydroxy-Pro, or N-Me-Ala;

A^3 = Pro, hydroxy-Pro, or N-Me-Ala;

A^5 and A^8 (independently) = Phe, thienylalanine, His, Trp, Nal, Pal, or P-X-Phe (X = F, Cl, Br, OH, or CH_3);

A^6 = Ser, Thr, Ala, Leu, Ile, Val, or Tyr;

A^7 = Pro, hydroxy-Pro, N-Me-Ala, D-Phe, or D-thienylalanine;

Z^{10} = OH, COOH, NH_2, or lower alkylamide;

provided that,

for each of the residues A^5, A^6, A^7, and A^8, independently, the carbon atom participating in the amide bond between that residue and the nitrogen atom of the alpha amino group of the adjacent amino acid residue may be a carbonyl carbon or may be reduced to a methylene carbon, provided that at least one such carbon atom must be reduced to a methylene carbon (i.e., at least one of the subject peptide CONH bonds must be replaced by a non-peptide CH_2NH bond); or a pharmaceutically acceptable salt thereof.

Preferred bradykinin analogs of the invention have non-peptide bonds joining residue A^8 to A^9, or A^5 to A^6. (In the examples given below, non-peptide bonds are symbolized by "$\psi[CH_2NH]$".)

Preferred analogs are:

Arg-Pro-Pro-Gly-Phe-$\psi[CH_2NH]$-Ser-Pro-Phe-Arg; and Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-$\psi[CH_2NH]$-Arg-OH.

The bradykinin analogs of the invention which act as bradykinin antagonists are useful for preventing increased vascular permeability that occurs during non-infectious inflammation, as analgesics, for preventing edema due to pulmonary brain trauma, in preventing shock due to hemorrhage, or for any condition characterized by vasodilation-induced swelling and/or itching.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

Description of the Preferred Embodiments

We now describe the structure, synthesis, and use of the preferred embodiments of the invention.

Structure

The bradykinin analogs of the invention all have a non-peptide bond in at least one of the indicated positions. By non-peptide bond is meant that the carbon atom participating in the bond between two residues is reduced from a carbonyl carbon to a methylene carbon. The peptide bond reduction method which yields this non-peptide bond is described in Coy et al., U.S. Patent No. 4,803,261, hereby incorporated by reference.

The peptides of the invention can be provided in the form of pharmaceutically acceptable salts. Examples of preferred salts are those with therapeutically acceptable organic acids, e.g., acetic, lactic, maleic, citric, malic, ascorbic, succinic, benzoic, salicylic, methanesulfonic, toluenesulfonic, or pamoic acid, as well as polymeric acids such as tannic acid or carboxymethyl cellulose, and salts with inorganic acids such as the hydrohalic acids, e.g., hydrochloric acid, sulfuric acid, or phosphoric acid.

Synthesis of bradykinin antagonists

The synthesis of Boc-Arg(tosyl)Pro-Pro-Gly-Phe-$\psi[CH_2NH]$-Ser(benzyl)-Pro-Phe-Arg(nitro)-O-resin is carried out as follows.

Boc-Arg(nitro)-polystyrene resin (Vega Biochemicals) (0.86 gm, 0.5 mmole) is placed in the reaction vessel of an Advanced ChemTech ACT 200 peptide synthesizer programmed to perform the following reaction cycle: (a) methylene chloride wash; (b) 33% trifluoroacetic acid in methylene chloride (2 times for 1 and 25 min each); (c) methylene chloride wash; (d) 10% triethylamine in dimethyformamide; (e) methylene chloride wash.

The neutralized resin is stirred with Boc-Phe and diisopropylcarbodiimide (1.5 mmole each) in methylene chloride for 1 h and the resulting amino acid resin is then cycled through steps (a) to (e) in the above wash program. The Boc group is then removed by TFA treatment and the following amino acid derivatives (1.5 mmole) are then coupled successively by the same procedure: Boc-Phe, Boc-Pro, Boc-Ser(benzyl). Boc-Phe aldehyde (1.5 mmoles), prepared by the method of Fehrentz and Castro, Synthesis, p. 676 (1983) is dissolved in 5 ml of dry DMF and added to the resin TFA salt suspension followed by the addition of 400 mg (8 mmoles) of sodium cyanoborohydride (Coy et al., U.S. Patent No. 4,803,261). After stirring for 1 h, the resin mixture is found to be negative to ninhydrin reaction (1 min) indicating complete derivitization of the free amino group.

After removal of the Boc group, Boc-Gly-p-nitrophenyl ester (3.0 mmoles) is then coupled in dimethylformamide. The following amino acids (1.5 mmole) are then coupled successively by the carbodiimide procedure: Boc-Pro, Boc-Pro, Boc-Arg(nitro). After drying, the peptide resin weighs 1.23 g.

The synthesis of H-Arg-Pro-Pro-Gly-Phe-ψ [CH_2NH]-Ser-Pro-Phe-Arg-OH follows.

The resin, as described above, (1.23 g, 0.5 mmole) is mixed with anisole (5 ml) and anhydrous hydrogenfluoride (35 ml) at 0°C and stirred for 45 min. Excess fluoride is evaporated rapidly under a stream of dry nitrogen and free peptide precipitated and washed with ether. The crude peptide is dissolved in a minimum volume of 2M acetic acid and eluted on a column (2.5 x 95 cm) of Sephadex G-25. Fractions containing a major

component by uv absorption (254 nm) and thin layer chromatography are then pooled, evaporated to a small volume and applied to a column (1.5 x 50 cm) of Vydac octadecylsilane (10-15μM).

The peptide is eluted with a linear gradient of 10-35% acentonitrile in 0.1% trifluoroacetic acid in water. Fractions are examined by thin layer chromatography and analytical high performance liquid chromatography and pooled to give maximum purity. Repeated lyophilization of the solution from water gives 104 mg of the product as a white, fluffy powder.

The product is found to be homogeneous by hplc and tlc. Amino acid analysis of an acid hydrolysate confirms the composition of the octapeptide. The presence of the Pheψ[CH_2NH] peptide bond is also demonstrated by fast atom bombardment mass spectrometry.

Other peptides can be prepared in similar yields in an analogous fashion by appropriately modifying the above procedure.

A linear bradykinin analog can be tested for effectiveness as either an agonist or antagonist of bradykinin using the following method.

Bradykinin-Stimulated Cyclic GMP Formation

Described below is a method that employs cultured neuroblastoma cells, which have high-affinity receptor sites for bradykinin and respond to bradykinin with an increase in intracellular levels of cyclic GMP.

Murine neuroblastoma cells (clone N1E-115) were cultured in Dulbecco's modified Eagle's medium without antibiotics and supplemented with 10% (vol/vol) fetal calf serum. Cells were grown in wells of a 24 multiwell plastic tray in an atmosphere of 10% CO_2-90% air at 37°C.

The cells were preincubated for 10 min. at 37°C in the presence of an antagonist of the invention and then incubated for 30 sec. with 30 nM bradykinin in a total volume of 0.20 ml. The reaction was terminated by aspiration of the incubation mix and the cells in each well were extracted with 0.8 ml of 2/3-1/3 (vol/vol) mixture of 95% ethanol-5 mM EDTA. The extracts were centrifuged and the supernatants were evaporated under a gentle stream of nitrogen. The extracts were then reconstituted in 50 mM EDTA-4 mM EDTA buffer and the cyclic GMP concentrations were measured by radioimmunoassy (cyclic GMP RIA kit, Amersham Corp).

Incubation of N_1E-115-cells with bradykinin produced a dose-dependent stimulation of cyclic GMP formation. Maximum stimulation of cyclic GMP formation (to ~ 6 pmoles) occurred at approximately 100 nM of bradykinin. Inhibition of bradykinin-stimulated cyclic GMP formation (to ~ 2.4 pmoles) by the bradykinin analogue, $Phe^5\psi[CH_2NH]$-bradykinin occurred at approximately 100 nM. The Phe^5-bradykinin antagonist is considerably more potent than other analogues, such as those developed by Stewart (Schachter et al., 1987, Br. J. Pharmac. 92:851-855; Rifo et al., 1987, Eur. J. Pharmac. 142:305-312; Vavrek and Stewart, 1985, Peptides 6:161-164; Steranka et al., 1987, Eur. J. Pharmac, 136:261-262).

Use

The peptides of the invention may be administered to a mammal, particularly a human, in one of the traditional modes (e.g., orally, parenterally, transdermally, or transmucosally), in a sustained release formulation using a biodegradable biocompatible polymer, or by on-site delivery using micelles, gels and liposomes.

The bradykinin analogs of the invention which act as antagonists are suitable for the treatment of medical disorders characterized by unwanted vascular dilation or permeability, e.g., common cold symptoms, vascular permeability-induced pain, edema caused by brain trauma, hemorrhage-induced shock, poison ivy, or other non-infectious swelling or itching. The peptides can be administered (most preferably, topically) to a human patient in a dosage of 0.5 µg/kg day to 5 mg/kg/day, preferably 10-1000 µg/kg/day.

Other embodiments are within the following claims.

Claims

1. A bradykinin analog of the formula:

$$Q\text{-}A^1\text{-}A^2\text{-}A^3\text{-}Gly\text{-}A^5\text{-}A^6\text{-}A^7\text{-}A^8\text{-}A^9\text{-}Z^{10}$$

wherein

Q =	L-Arg, D-Arg, L-homo-Arg, D-homo-Arg, L-Lys, D-Lys, lower (1-5 carbon atoms) Σ-N-alkyl-L-Lys, lower Σ-N-alkyl-D-Lys, lower Σ-N-alkyl-L-His, lower Σ-N-alkyl-D-His, lower Σ-N-alkyl-L-Pal, lower Σ-N-alkyl-D-Pal, acetyl, lower acyl, or lower α-N-alkyl;
A^1 and A^9 (independently) =	Arg, homo-Arg, Lys, lower Σ-N-alkyl-Lys, His, or Pal;
A^2 =	Pro, hydroxy-Pro, or N-Me-Ala;
A^3 =	Pro, hydroxy-Pro, or N-Me-Ala;
A^5 and A^8 (independently) =	Phe, thienylalanine, His Trp, Nal, Pal, or P-X-Phe, (X = F, Cl, Br, OH, or CH_3);
A^6 =	Ser, Thr, Ala, Leu, Ile, Val, or Tyr;
A^7 =	Pro, hydroxy-Pro, N-Me-Ala, D-Phe, or D-thienylalanine;
Z^{10} =	OH, COOH, NH_2, or lower alkylamide;

provided that,

for each of the residues A^5, A^6, A^7, and A^8, independently, the carbon atom participating in the amide bond between that residue and the nitrogen atom of the alpha amino group of the adjacent amino acid residue may be a carbonyl carbon or may be reduced to a methylene carbon, provided that at least one such carbon atom must be reduced to a methylene carbon.

2. The Bradykinin analog of claim 1 wherein, for each of the residues A^5 or A^8, independently, the carbon atom participating in the amide bond between that residue and the nitrogen atom of the alpha amino group of the adjacent amino acid residue may be a carbonyl carbon or may be reduced to a methylene carbon, provided that at least one such carbon atom must be reduced to a methylene carbon .

3. The bradykinin analog of claim 1 having the amino acid formula

Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-$\psi[CH_2NH]$-Arg-OH.

4. The bradykinin analog of claim 1 having the formula:

Arg-Pro-Pro-Gly-Phe-$\psi[CH_2NH]$-Ser-Pro-Phe-Arg-OH.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US 89/01216

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC (4): C07K 7/18
U.S. Cl: 530/314,328,334

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S.	530/314,328,334

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
P,X	European Journal of Pharmacology, Vol. 155, issued 1988, Drapeau, "[Phe$^{8}\psi$(CH_2-NH) Arg^{9}] bradykinin, a B_2 receptor selective agonist which is not broken down by either kininase I or Kininase II", pp. 193-195, see the entire article.	1-4
Y	J. Med. Chem., Vol. 28, issued 1985. Martinez, "Synthesis and Biological Activities of some pseudo-peptide analogues of tetragastrin. The importance of the peptide backbone", pp. 1874-1879, See the entire article.	1-4
A	J. Med. Chem., vol. 30, issued 1987, Sasaki, "Solid-phase synthesis and biological properties of ψ[CH_2NH] pseudopeptide Analogues of a highly potent somatostatin octapeptide", pp. 1162-1166, See the entire article.	1-4
A,P	US,A, 4,803,261, 07 February 1989. (COY) See the entire document.	1-4

* Special categories of cited documents: [10]

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"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
05 June 1989	27 JUL 1989
International Searching Authority	**Signature of Authorized Officer**
US/ISA	T.D. Wessendorf

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III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
A	EP,A, 0,045,665, 10 February 1982, (SZELKE). See the entire document.	1-4
Y	CHEMICAL ABSTRACTS, Vol. 107, No.7, issued 1987 (Columbus, Ohio, USA), Rodriquez, "Synthesis and biological activities of pseudopeptide analogs of the C-terminal heptapeptide of cholecystokinin. On the importance of the peptide bonds", see page 778, col. 2, abstract no 59451d J. Med. Chem., 1987, 30(8), 1366-73(Eng).	1-4
A	US,A, 3,422,083, 14 January 1969,(HESS) See the entire document.	1-4